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The Influence of the Stage of Maturity on the Chemical Composition and the Vitamin B (B_1) and G Content of Hays and Pasture Grasses

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INTRODUCTION

The importance and extensive use of hays and pasture crops in the rations of livestock make imperative fundamental knowledge concerning the total nutritive value of these products and the factors that affect their quality, in order that their value and place in a feeding program may be determined. In the past, hays were produced largely with one object in mind; namely, that of quantity or total yield. Very little thought was given to the quality of pastures and their effect on livestock production. More recently, attention has been directed to the factors that affect the nutritive properties of hays and pasture crops, with the object of producing a product having the maximum amount of feeding value.

Many investigators have pointed out the importance of these products as a source of minerals and vitamins for growing and producing animals, but the more recent investigations have in the main dealt only with the vitamins A and D.

Alfalfa leaf meal and alfalfa meal are used extensively in poultry rations, in part for their vitamin G[1] content. Pasture crops are extensively used in the swine, sheep, and cattle industry to produce fast gains and economy in production. There is no doubt that all of the vitamins, as well as the minerals, play a part in the well-being of the animal; yet no information is available regarding the vitamin G content of hays and pasture crops and the factors that affect it. The present report in part covers a study of the chemical composition and the vitamin B_1 and G content of alfalfa, clover, and timothy hays and bluegrass and wheat pasture grass as they are influenced by the stage of maturity of the plant when cut and natural climatic factors. It also includes the results of growth experiments with chicks, for the purpose of studying their vitamin G requirement, as measured by the rat unit method.

REVIEW OF THE LITERATURE

The literature contains data supporting the view that certain practices or procedures affect the vitamin content of hays and pasture grasses. Steenbock, Hart, and associates (34) found that clover hay which had been exposed to sunlight and rain was less valuable as a source of vitamin A but that it had better calcifying properties than hay which had been cured quickly and still retained a good green color. Russell (32) has confirmed the above work by showing that alfalfa hay dried artificially was about seven times as potent in vitamin A as hay cured in the usual way, although Kieselbach and Anderson (24) found no significant difference in vitamin A content between field cured and artificially cured alfalfa, when poultry were the experimental animals.

[1]Vitamin G as here used has reference to the complex and not to any of the individual factors.

Russell (32) also noted an increase in the vitamin D content when alfalfa hay was cured in the sun. Unpublished data obtained at the Ohio Station have confirmed the above results. Bethke and Kick (3) observed a loss of vitamin A in alfalfa hay cured in the sun and exposed to dew and rain. Hauge and Aitkenhead (18) also confirmed the foregoing results and explained that the destruction of vitamin A was due to an enzyme. Hathaway, Davis, and Graves (17) found that artificially cured alfalfa hay was twice as potent in vitamin A as field cured alfalfa. Hartman (16) determined the vitamin A content of different grades of alfalfa and timothy hays and found that this vitamin varied according to the method of curing. Douglas, Tobiska, and Vail (8) have reported that alfalfa cut at the early bloom stage contains more vitamin A and G than hay cut at a later stage of maturity. They also presented data which showed that rain and sunshine lowered the vitamin A and B_1 content and possibly the vitamin G content. Hunt and Krauss (21) in a study of pasture grasses found that the vitamin G content decreased as the plant matured.

It is very evident from the data obtained in the investigations reviewed that the stage of maturity of the plant when cut and natural climatic influences are very important factors in determining the quality of hays (in terms of vitamins) and should be taken into consideration when making hay.

It has only been in the past few years that much attention has been given to the influence of feed upon the animal to which it was fed and upon the quality of products produced by the animal. However, Funk (10) about 22 years ago suggested that the vitamin content of milk was influenced by the quality of the feed of the cow. A little later McCollum and coworkers (29) presented evidence that the ration of the lactating animal influenced the vitamin A and B (complex) content of milk, and Steenbock and associates (33) noted a variation in the vitamin content of butter and surmised that this variation was due to the feed of the cow. Barnes and Hume (1) and Dutcher and coworkers (9) had shown also that milk from cows on pasture had a higher vitamin C content than milk from cows on winter feed, although Hughes and associates (20) have since then obtained data which were not in accord with the above results. Kennedy and Dutcher (23) found that the vitamins A and B (complex) in milk are regulated by the diet, but more recently Gunderson and Steenbock (14), after attempting to increase the vitamin B (complex?) content of cow's milk, have concluded that the maximum content of this vitamin is under physiological control.

In 1923 Golding (12) reported results which show that winter feeding, as practiced in parts of England, may reduce the vitamin A value of milk to one-tenth of its summer value. He stated further that neither dry nor parched pastures could compare with the value of fresh green grass as a source of vitamin A. In a later investigation Golding and associates (13) found that kale when fed in the winter ration of the cow raised the vitamin A content but not the vitamin D content of the milk. Luce (27), after a study of the effect of the diet upon the vitamin content of milk, concluded that the growth promoting (vitamin A) and the antirachitic (vitamin D) value of milk depended upon the amount of these vitamins in the diet of the cow. Bethke, Kennard, and Sassaman (2) found that the fat soluble vitamins (A and D) of hens' eggs were greatly increased by the amount of these vitamins in the ration and the environment of the laying hen. Krauss (25) reported that cows on pasture for 16 days produced butterfat with twice the vitamin A potency of that obtained from the same cows before going on pasture. Hunt and Krauss (21) obtained data which showed that cows on pasture produced milk of a relatively high vitamin G content and that, as the pasture matured, the vitamin G content of the milk was lowered. The vitamin B_1 content of the milk was not so affected.

Bethke and Record (4) have shown that vitamin G, as found in dried liver, autoclaved yeast, and dried whey, exerted a beneficial effect on growth response in chicks and on hatchability of eggs. In 1932 Krauss and Hayden (26) found a difference in the vitamin A and D content of milk and concluded that the difference might be due to the vitamin content of the feed. Hilton and associates (19) have shown that timothy produced butter of low vitamin A value whereas good alfalfa and soybean hays were effective in producing butter of as high a vitamin A value as summer pasture. Hart and Guilbert (15) found that cattle may show the effect of a vitamin A deficiency if their feed is obtained from a dry range for 3 or 4 months. The vitamin A potency of the grass on the range was found to be associated with the degree of greenness. Meigs and Converse (28) concluded from their studies that, when the roughage consisted of timothy hay of mediocre or poor quality, the milk yield was reduced and the health and staying powers of the cows and the calving were less satisfactory than those on the alfalfa rations. They were of the opinion that the results could be partially explained on the basis of the fact that inferior timothy hay is low in vitamin A; Jacobson (22) is of the same opinion with reference to prairie hay, when judged by production and reproduction in cattle.

It is realized that other factors than vitamins are essential in animal nutrition and that abnormal effects appear in animals when they are absent or reduced in amount through the feeding of plant products produced under unfavorable conditions. A rather comprehensive review of this phase of research is given in Ohio Experiment Station Bulletin 415.

SOURCE OF HAYS

The alfalfa and clover hays used in this investigation were either grown on the Station farm or obtained from the Department of Farm Crops, the Ohio State University. The timothy samples were either grown on the Station farm or on plots of the U. S. Department of Agriculture, Bureau of Plant Industry, Timothy Breeding Station, North Ridgeville, Ohio. The bluegrass and wheat grass were grown on the Station farm. The bluegrass plots were located on fertile soil but were not fertilized. Unless otherwise stated, the samples were cured indoors in subdued light.

RAT EXPERIMENTS

Rats were used as the experimental animals for evaluating the hays as to vitamin content. These animals were born to mothers on a modified Steenbock stock ration (5). When 24 days of age or when weighing from 45 to 60 grams, the young were placed in screen bottom cages and were given a vitamin B– and G–free ration. This diet consisted of casein (extracted), 18; corn starch, 64; hydrogenated fat (Crisco), 10; agar, 2; cod-liver oil, 2; and salt mixture (185), 4.

If the rats were to be used for the determination of vitamin B (B_1), they were fed 400 milligrams of yeast (Northwestern), autoclaved at 15 pounds pressure for 4 hours, per rat daily in addition to the basal diet. When the animals had ceased gaining in weight (the period required was from 14 to 21 days) they were placed in individual wire cages and fed daily the hay supplemented with 400 milligrams of autoclaved yeast. The supplement was moistened with distilled water to prevent scattering.

For the determination of vitamin G, the basal diet was supplemented with 0.5 cubic centimeter of an extract of rice polishings per rat daily to supply vitamin B_1. When the weight of the animals became constant or they had slightly lost in weight, they were transferred to individual wire cages and the supplement (hay), moistened with 1 cubic centimeter of the vitamin B_1 preparation, fed daily. The depletion period required on the average about 24 days.

The vitamin B_1 extract was prepared as follows:[2] Four kilos of rice polishings were shaken up with 8 liters of 0.1 per cent HCl. After the mixture had stood over night, the clear supernatant liquid was siphoned off. This was repeated five or six times and the extracts combined and evaporated before an electric fan to 1200 or 1400 cubic centimeters and then made up to 80 per cent alcohol by weight. The precipitate was filtered off. The alcohol from the filtrate was partially removed by distillation in a partial vacuum and the last traces before an electric fan. The residual liquid was then diluted with water to the desired concentration (1 cubic centimeter = 1 gram rice polishings).

The hays and pasture crops were ground to a fine flour in a ball mill for feeding purposes. The experimental feeding period in all cases was of 6 weeks' duration. The results are expressed in terms of rat units as outlined by Bourquin and Sherman (6) and Chase and Sherman (7).

RESULTS

The results of the chemical analyses as presented in Table 1 show that the protein content of the plant decreases and the crude fiber increases as the plant matures. That this is due to the decreased leaf content is clearly shown in Samples 1 and 3, Table 1. The attack of leaf hoppers no doubt lowered the leaf content and, hence, the protein content, as is shown in Sample 6. Unpublished data from the Ohio Station show that second cutting alfalfa not attacked by the leaf hopper may contain 18 per cent of protein.

RAT EXPERIMENTS

The results of the biological analyses for vitamin B_1, presented in Table 2, show that hays are comparatively low in this factor. Alfalfa (bud stage, May 31) appears to have a higher vitamin B_1 content than either clover or timothy cut at approximately the same stage of maturity (June 10 and 12, respectively). The data also suggest that the vitamin B_1 content of the plant decreases as it matures.

The results of the vitamin G assays (Table 3) show that hays may serve as a good source of this factor. In general, the vitamin G content is highest in the young, immature plant and decreases as the plant matures. The relation of the maturity of the plant to its vitamin G content is particularly evident in the case of alfalfa and timothy. The results with clover, on the contrary, do not show this relationship. Apparently, in the case of the clover, the time elapsing between the two cuttings has not been sufficiently long to bring out the comparison of the more or less immature and mature plant. It is also

[2]This is now prepared by making a 95 per cent alcoholic extract of rice polishings. The alcohol is removed by vacuum distillation and then taken up with water (acidified).

of interest to note that clover and timothy may have as high a vitamin G (complex) content as alfalfa when the respective plants are harvested at corresponding stages of maturity. The comparison between alfalfa and timothy is readily seen in the case of the samples grown as mixed hays.

TABLE 1.—Chemical Composition of Hays

No.	Sample	Date cut	Moisture	Ash	Nitrogen	Protein N×6.25	Ether extract	Crude fiber	Nitrogen-free extract	Remarks
			Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	
1	Alfalfa ..	May 14	8.08	8.11	3.57	22.31	3.01	18.58	39.91	Leaves–60 pct. Stems–40 pct.
2	Alfalfa ..	May 31	7.67	6.92	3.22	20.13	2.18	25.27	37.83	Crop 1932.
3	Alfalfa ..	June 10	7.12	7.21	2.21	13.81	3.04	30.02	38.80	Leaves–40 pct. Stems–60 pct.
4	Alfalfa ..	June 10	6.66	6.17	2.82	17.62	2.05	32.41	35.09	Grown on same soil as No. 2, 1932.
5	Alfalfa ..	June 21	7.23	5.47	2.49	15.57	1.48	34.76	35.49	Crop 1932.
6	Alfalfa ..	July 28	9.15	5.96	2.07	12.94	1.63	32.51	37.81	Crop 1932. Second cutting. Leaf-hopper injury.
7	Alfalfa ..	Sept. 10	8.51	7.70	3.19	19.93	2.73	23.49	37.64	Third cutting, crop 1932.
8	Alfalfa ..	....	11.90	6.89	2.86	17.87	2.45	23.11	37.78	Open market.
9	Alfalfa ..	....	10.28	9.24	2.73	17.05	1.80	23.24	38.39	Western product.
10	Clover...	June 10	7.29	6.19	2.51	15.69	3.18	23.30	44.35	Crop 1932.
11	Clover...	June 21	6.94	4.99	1.96	12.25	1.99	28.73	45.10	Crop 1932.
12	Timothy.	June 12	6.95	7.07	1.36	8.50	2.08	25.92	49.48	Heads emerging. Crop 1931.
13	Timothy.	June 18	8.73	5.50	1.06	6.62	2.07	25.65	51.43	Heads out. Crop 1932.
14	Timothy.	June 24	6.87	6.86	1.13	7.06	3.08	28.78	47.35	Early bloom. Crop 1931.
15	Timothy.	July 1	8.95	4.67	0.83	5.19	1.90	26.97	52.32	Late bloom. Crop 1932.
16	Timothy.	July 13	6.10	5.92	0.84	5.25	1.31	30.06	51.36	Straw color. Crop 1931.
17	Timothy No. 15150	June 18	7.33	6.32	0.90	5.62	2.43	29.44	48.86	Late maturing. Heads emerging.
18	Timothy No. 12368	July 1	7.77	5.45	0.84	5.25	2.04	31.76	47.73	Late maturing. Early bloom.
19	Timothy.	June 8	9.74	6.89	1.39	8.68	2.15	23.80	48.74	Grown as mixed timothy and alfalfa.
20	Alfalfa ..	June 8	11.15	7.00	2.52	15.75	1.80	25.95	38.35	Grown as mixed timothy and alfalfa.
21	Mixed sample about 50:50....	June 8	8.96	6.20	1.63	10.18	2.31	24.23	48.12	Grown as mixed timothy and alfalfa.

Leaf-hopper injury of alfalfa reduces its vitamin G content; this reduction no doubt is primarily due to the loss of leaves. Exposure of alfalfa and clover to weather (day and night) for 96 hours, more than one-half of which was sunshine, seems not to have affected the vitamin G content of the hay. Rain (0.68 inch), on the contrary, has lowered the vitamin G content by 50 per cent, as shown in the case of alfalfa (5), Table 3.

Table 4 shows the relationship between the protein and the vitamin B_1 and G content of hays. It will be observed that, in general, a high protein content is correlated with a high vitamin G content. It is well known that as the plant matures the proportion of leaves to the entire plant decreases, and consequently

TABLE 2.—Vitamin B_1 Value of Hays

Sample	Date cut	No. of rats	Level fed daily	Total gain	Weekly gain	Vitamin B_1, units per gm.*	Remarks
			Mg.	*Gm.*	*Gm.*		
Alfalfa (a)†	May 31, 1932	8 8	350 450	11.7 22.3	1.9 3.7	2.5	University farm
	June 10, 1932	8 8	400 500	15.3 18.1	2.5 3.0	2.0	
	June 21, 1932	8 8	500 600	8.1 18.3	1.5 3.0	1.6	
	July 28, 1932	8 8	400 600	15.8 22.3	2.6 3.7	2.0	Second cutting. Leafhopper injury.
	Sept. 10, 1932	8 8	450 500	13.3 18.2	2.2 3.0	2.0	Third cutting.
Alfalfa (b)	June 8, 1932	8 8	400 500	19.0 26.0	3.1 4.3	2.5	Station farm
Timothy (c)	June 12, 1931	8	600	7.2	1.2	0.7—0.8	Bureau of Plant Industry
	June 24, 1931	8	700	5.1	0.85	0.6‡	
	July 13, 1931	8	600	-2.0	-0.33	0.5‡	
	June 18, 1932	8 8	700 900	11.1 21.8	1.8 3.6	1.2‡	
Timothy (c) No. 15150	June 18, 1932	8 8	600 800	12.5 23.8	2.0 3.9	1.4	Late maturing. Bureau of Plant Industry
Timothy (c) No. 12368	July 1, 1932	8	600	6.5	1.1	0.5‡	
Timothy (c)	July 1, 1932	8	1000	11.5	1.9	0.6‡	Regular
Clover (a)	June 10, 1932	8 8	500 600	13.1 22.3	2.2 3.7	1.7	University farm
	June 21, 1932	8 8	500 600	13.1 27.2	2.2 4.5	1.6-1.8	
Alfalfa from alfalfa and timothy mixture (b)	June 8, 1932	8 8	400 600	6.6 22.2	1.1 3.7	1.6	Grown as a mixed hay. Station farm
Timothy from alfalfa and timothy mixture (b)	June 8, 1932	8 8	600 800	11.4 24.5	1.9 4.1	1.2	
Alfalfa and timothy mixture (50:50) (b)	June 8, 1932	8 8	650 700	16.7 21.3	2.8 3.5	1.4	
Controls		18	400 mg. autoclaved yeast				All died in 3 weeks

*A unit of vitamin B_1 or G is the weight of the supplement which, when fed to rats under standard conditions, will produce an increase in weight of 3 to 4 grams per week.

†Same number indicates hays were grown in same or adjacent fields.

‡Estimated—animal could not consume sufficient hay to produce the required increase in weight.

TABLE 3.—Vitamin G Value of Hays

Sample	Date cut	No. of rats	Level fed daily	Total gain	Weekly gain	Vitamin G units per gm.	Remarks
			Mg.	*Gm.*	*Gm.*		
Alfalfa (a)	May 31, 1932	8 8	100 150	25.1 40.8	4.2 6.8	10–12	University farm
	June 10, 1932	8	100	24.9	4.1	10.0	
	June 21, 1932	8 8	150 200	27.4 34.8	4.5 5.8	6.6	
	July 28, 1932	8	150	24.0	4.0	6.6	University farm Second cutting. Leafhopper injury
	Sept. 10, 1932	8	75	22.6	3.8	13.0	University farm Third cutting
Alfalfa (b)	June 8, 1932	8 8	100 150	15.9 16.7	2.6 2.8	6.6	Station farm
	July 21, 1932	8	100	18.5	3.0	10.0	Second cutting, sun cured. No rain
		8	200	20.7	3.4	5.0	Second cutting, sun cured. 0.68 in. rain
Timothy (c)	June 12, 1931	8 8	100 150	15.0 24.0	2.5 4.0	6.6	Heads emerging
	June 24, 1931	8 8	150 300	14.2 25.4	2.3 4.2	3–4	Full bloom
	July 13, 1931	8 8	250 400	13.0 25.0	2.2 4.1	2.5	Ripe brown color
	June 18, 1932	8 8	150 300	9.7 19.7	1.6 3.3	3.3	Early bloom
	July 1, 1932	8 8	300 450	17.4 24.7	2.9 4.0	2–3	Late bloom
Timothy (c) No. 15150	June 18, 1932	8 8	150 300	7.5 16.0	1.2 2.6	2–3	Bureau of Plant Industry Late maturing variety
Timothy (c) No. 12368	July 1, 1932	8 8	150 300	9.4 18.0	1.5 3.0	3.3	Bureau of Plant Industry Late maturing variety
Timothy (c) No. 12321	June 20, 1933	8 8	100 125	16.0 19.9	2.6 3.3	8.0	Bureau of Plant Industry Late maturing variety Sun cured

TABLE 3.—Vitamin G Value of Hays—Continued

Sample	Date cut	No. of rats	Level fed daily	Total gain	Weekly gain	Vitamin G units per gm.	Remarks
			Mg.	*Gm.*	*Gm.*		
Clover (a)	June 10, 1932	8 8	100 150	24.9 28.7	4.1 4.8	10.0	University farm
	June 21, 1932	8 8	100 200	27.7 37.8	4.6 6.3	10.0	
	June 17, 1932	8 8	100 100	17.1 17.4	2.9 2.9	8.0 8.0	Cured indoors 96 hr. exposure. No rain
Alfalfa from alfalfa and timothy mixture (b)	June 8, 1932	8 8	100 150	16.0 21.3	2.7 3.5	6.6	Grown as mixed hay Station farm
Timothy from alfalfa and timothy mixture (b)	June 8, 1932	8 8	100 200	15.5 23.3	2.6 3.9	5.0	
Alfalfa and timothy mixture 50:50 (b)	June 8, 1932	8 8	100 200	16.4 29.0	2.7 4.8	6.6	
Controls		16		-2.0			

TABLE 4.—Chemical and Biological Analyses of Hays

Sample	Date cut	Crude fiber	Protein	Vitamin B_1 units per gm.	Vitamin G units per gm.	Remarks
		Pct.	*Pct.*			
Alfalfa (b)	May 14, 1931 June 10, 1931	17.42 30.02	22.32 13.83	2.5 1.8	13.0 6.6	60 per cent leaves 40 per cent leaves
Alfalfa (a)	May 31, 1932 June 10, 1932 June 21, 1932 July 28, 1932 Sept. 10, 1932	25.27 32.41 34.76 32.51 23.49	20.14 17.60 15.59 12.91 19.92	2.5 2.0 1.6 2.0 2.0	10–12 10.0 6.6 6.6 13.0	Early bud Early bloom Late bloom Second cutting Hopper injury Third cutting
Clover (a)	June 10, 1932 June 21, 1932	23.30 28.73	15.67 12.26	1.7 1.9	10.0 10.0	Early bloom Late bloom
Clover (b)	June 17, 1933	....	12.13	....	8.0	Sun cured
Timothy (c)	June 12, 1931 June 24, 1931 July 13, 1931 June 18, 1932 July 1, 1932	23.79 31.05 .... 25.65 26.97	8.50 7.25 5.00 6.65 5.21	0.7–0.8 0.6 0.5 1.1 0.6	6.6 3–4 2.5 3.3 2–3	Heads emerging Full bloom Brown (ripe) color Early bloom Late bloom
Timothy from alfalfa and timothy mixture	June 8, 1932	23.80	8.66	1.4	5.0	Grown as mixed hay
Alfalfa from alfalfa and timothy mixture	June 8, 1932	25.95	15.76	1.7	6.6	
Mixed alfalfa and timothy (about half and half)	June 8, 1932	24.23	10.20	1.4	6.6	

the protein and the vitamin B_1 and G decrease. Accordingly, any procedure or process that would reduce the leaf content of the hay, such as insect injury, excessive handling, or late cutting, would naturally lower the protein and vitamin content and also the quality of the hay.

The correlation of protein and vitamin G content with greenness is only relative and varies with the amount of bleaching due to light, the extent of insect injury (leaf hopper), and whether or not the plant is diseased. A similar greenness in alfalfa, clover, and timothy does not necessarily indicate the same vitamin G content. In general, however, the vitamin G values follow in the same order as the protein, leafiness, and greenness of the particular plant.[3]

The chemical and biological analyses of the forage crops under study are given in Table 5. The chemical analyses clearly demonstrate the influence of the stage of maturity on the protein content of bluegrass and wheat; that is, the protein content decreases as the plant matures. This agrees with McCreary's (30) work with forage crops of Wyoming.

In the case of pastures, the stage of maturity may be influenced by several factors, such as closeness of grazing, the length of time the animals are away from the pasture, and the rate of growth as it is influenced by the temperature, rainfall, and the fertility of the soil. Although complete data are not available, what are given must be considered in interpreting the results.

The protein content of early and late cut bluegrass is comparable to that of alfalfa cut at corresponding stages of maturity; the difference in the vitamin G content of the early and late cut bluegrass is in the same order but not quite so striking as in the case of alfalfa.

The data in Table 5 clearly show that bluegrass, wheat, and rye in the early stages of development compare favorably with alfalfa and clover, in similar stages of development, in their protein content as well as in their vitamin G content. Drying appears to have caused some destruction of vitamin G as found in wheat grass.

CHICK EXPERIMENTS

In order to obtain further data on the comparative vitamin G value of different alfalfa and alfalfa leaf meals, day-old chicks were used as the experimental animals. These experiments also gave a direct comparison of the chick and rat assay method for vitamin G.

White Leghorn chicks from the same parent stock were used. They were confined in pens equipped with hardware cloth floors and fed a ration known to be deficient in vitamin G. Each lot contained 25 chicks. The basal ration was composed of yellow corn, 58; ground wheat, 20; wheat bran, 5; casein (Argentine), 12; steamed bone meal, 3; salt, 1; and cod-liver oil, 1. The meals were incorporated in this ration in the amount indicated in Table 6. The casein and corn were adjusted so that the protein content was approximately the same in

[3]While the above data were being collected, numerous experiments were being reported on the effect of green grass and pasture crops on different species of animals. Robison (31) reports that 99 per cent of the pigs in a pasture group getting a grain and tankage ration completed the experiment in good condition; whereas 12 per cent of those not in a pasture group but getting a supposedly complete ration had to be removed from the experiment due to unthriftiness or other causes. Similar experiments (11) have been reported regarding the effect of green feed on the thriftiness of poultry, sheep, and cattle. We know that such an effect on poultry can be partially explained as due to the vitamin G content of the feed. However, no study has been made of the effect of vitamin G — deficient rations on sheep, hogs, and cattle, and whether the influence of the pasture crops is due to their vitamin G content remains to be determined.

The samples of bluegrass used in this study were collected and the physical data obtained by Mr. Robison.

TABLE 5.—The Chemical and Biological Analyses of Pasture Grasses

Material	Date grass was cut	Preceding cutting was made	Days between cuttings	Rainfall between cuttings	Height when cut	Moisture content of green material	Composition of dry matter				Moisture, air-dry basis	Units vitamin G per gm.
							Ash	Protein	Crude fiber	Ether extract		
				In.	*In.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	
Bluegrass	May 10	....	....	....	9	79.7	8.42	26.49	22.91	2.42	7.97	10.0
Bluegrass	May 25	....	....	....	12	70.3	6.79	18.89	22.17	3.44	8.38	5.0- 6.6
Bluegrass	June 9	....	....	....	15	78.0	7.85	16.83	23.21	3.68	8.28	6.6
Bluegrass	June 26	....	....	....	16	60.4	8.47	16.60	20.95	3.13	9.26	6.6
Bluegrass	July 31	June 9	52	6.6	5	61.1	8.36	21.09	20.63	....	9.99	5.0- 6.6
Bluegrass	July 31	June 26	35	4.7	5	63.1	7.17	20.27	19.36	....	11.43	6.6
Bluegrass	Aug. 27	July 31	27	4.2	8	79.4	9.60	25.41	....	....	6.28	12.0
Bluegrass	Oct. 1	Aug. 27	35	6.2	9	79.1	10.35	23.33	....	....	5.70	12.0
Bluegrass	Oct. 1	July 31	62	10.4	18	80.3	9.49	19.12	....	....	6.18	10.0
Bluegrass	Oct. 31	Oct. 1	30	1.0	4	77.9	12.16	26.32	....	....	6.22	10.0
Rye—ready to head	May 18	....	....	....	12	83.5	9.75	24.51	19.64	4.04	10.24	8.0
Winter wheat, spring seeded	July 3	....	....	....	....	79.3	11.01	26.28	18.61	5.34	8.23	12.0
Wheat grass	1935 May 1	....	....	....	8–10	....	11.78	24.14	....	....	8.38	8.0
Wheat grass	1935 May 13	....	....	....	12–15	....	11.51	21.52	....	....	7.94	5.0- 6.6
Wheat grass	1936 May 1	....	....	....	8–10	....	10.27	22.83	....	....	9.45	11.0
Wheat grass	1936 May 1	....	....	....	8–10	83.09	....	....	....	....	....	2.6

TABLE 6.—Effect of Alfalfa, Alfalfa Leaf, and Liver Meals on Growth and Leg Paralysis of Chicks

Lot No.	Supplement	Protein in meals	Meals consumed per chick in 8 weeks	Rat units vitamin G per gm. meal	Total rat units vitamin G consumed per chick in 8 weeks	Average weight per chick in 8 weeks	Leg paralysis	
							Total*	Recovered†
	Pct.	Pct.	Gm.			Gm.	Pct.	Pct.
1	None	....	....	....	....	168.8± 4.8	67.0	0.0
2	5—alfalfa meal‡	16.0	38.7	8.0	309.6	278.8±14.0	68.0	8.0
3	10—alfalfa meal‡	16.0	118.5	8.0	948.0	466.8±13.3	29.0	17.0
4	5—alfalfa leaf meal‡	20.25	49.9	13.0	648.7	384.6±15.2	48.0	12.0
5	10—alfalfa leaf meal‡	20.25	130.6	13.0	1697.8	517.3±11.8	0.0	....
6	5—alfalfa meal§	14.25	35.6	8.0	284.8	251.9±11.3	87.0	35.0
7	10—alfalfa meal§	14.25	119.7	8.0	957.6	454.9±15.0	28.0	16.0
8	5—alfalfa leaf meal§	20.56	55.2	13.0	717.6	438.3±14.4	48.0	48.0
9	10—alfalfa leaf meal§	20.56	132.4	13.0	1721.2	528.3± 9.9	0.0	....
10	5—alfalfa leaf meal (0.68 in. rain)‡	20.31	32.8	5.0	164.0	240.6±11.9	83.0	33.0
11	10—alfalfa leaf meal (0.68 in. rain)‡	20.31	107.1	5.0	535.5	397.9±13.6	36.0	20.0
12	3—dried pork liver	....	....	....	....	602.9±12.3	0.0	....

*The per cent of the total number that developed leg paralysis during the experiment.
†The per cent of the total number that recovered before the end of the experiment.
‡Meals from hays grown in Ohio.
§Meals from hays grown in Colorado.

all lots. The meals used represented composite samples of three or more lots, with the exception of the Ohio leaf meal, which involved only one sample. The Ohio leaf meal fed to Lots 10 and 11 (Table 6) was prepared from hay that was cured in the sun and that had received 0.68 inch of rain while in the swath (see Table 3—alfalfa (b) cut July 21). Liver meal is considered a very rich source of vitamin G, and for that reason one lot of chicks was fed 3 per cent of this in the basal ration as a positive control.

It is evident from the data presented in Table 6 that the growth of the chicks and the incidence of leg paralysis are directly correlated with the vitamin G content of the meals as determined with rats. Leaf meals having a higher protein and vitamin G content than straight meals caused greater growth and less incidence of leg paralysis than regular meals of lower protein and vitamin G content. The poor growth and comparatively large percentage of leg paralysis in the case of Lots 10 and 11, which received 5 and 10 per cent, respectively, of the leaf meal subjected to rain, suggest that a large part of the vitamin G had been lost and corroborate the results obtained with rats. The data also clearly show that alfalfa meals and leaf meals of approximately the same vitamin G potency produce comparable responses in chicks, irrespective of whether they are produced in Ohio or Colorado.

A second experiment was conducted to check the results of the first trial and to compare meals prepared from clover and timothy with those from alfalfa. The same basal ration and procedure as used in the first test were employed. The meals were incorporated in the basal ration in amounts shown in Table 7. The casein and corn were adjusted so that the total protein content of the rations in all lots was comparable. The clover and timothy meals were prepared from single samples of hay; the alfalfa meals represented composite samples of three or more lots.

The results presented in Table 7 show the ineffectiveness of 10 per cent of the straight hay meals in preventing leg paralysis—substantiating the results obtained in the first experiment. Although the results show some variation among different meals, the data, in general, reveal a close correlation between the response in growth and incidence of leg paralysis and the vitamin G content of the meals as determined on rats. Clover meal gave results comparable to alfalfa meals of the same vitamin G potency but a higher protein content. The results obtained with the timothy meals also compare favorably with those of alfalfa when the vitamin G content of the meals is taken into consideration. As in the previous experiments, there was no difference in response between Ohio and Colorado meals when compared on the same vitamin G basis.

The data in Tables 6 and 7 clearly show that there is a close correlation between the total consumption of vitamin G and the weight of the chick and the incidence of leg paralysis.

From the evidence obtained by the method used in this investigation, it appears that 10 per cent of an alfalfa leaf meal containing 13 rat units of vitamin G per gram is necessary to induce good growth and prevent the occurrence of leg paralysis.

TABLE 7.—Effect of Alfalfa, Clover, Timothy, and Liver Meals on Growth and Leg Paralysis of Chicks

Lot No.	Supplement	Protein in meals	Meals consumed per chick in 8 weeks	Rat units vitamin G per gm. meal	Total rat units vitamin G consumed per chick in 8 weeks	Average weight per chick in 8 weeks	Leg paralysis	
							Total*	Recovered†
	Pct.	*Pct.*	*Gm.*			*Gm.*	*Pct.*	*Pct.*
1	None	....	....	....	....	176.0± 5.3	95.0	15.0
2	5—clover‡	11.54	38.0	8.0	304.0	243.0±12.3	85.0	35.0
3	10—clover‡	11.54	125.7	8.0	1005.6	453.5±20.2	15.5	0.0
4	5—timothy§	7.63	38.9	5.0	194.5	261.8± 9.0	65.0	5.0
5	10—timothy§	7.63	100.2	5.0	501.0	345.5±11.8	70.0	45.0
6	10—timothy‖	5.62	92.3	3.0	276.9	314.0±10.6	70.0	25.0
7	5—alfalfa meal**	12.25	48.2	8.0	385.6	306.0±11.1	80.0	35.0
8	10—alfalfa meal**	12.25	110.8	8.0	886.4	389.8±13.2	40.0	5.0
9	10—alfalfa meal***	14.25	101.1	6.6	667.2	368.7±11.9	45.0	35.0
10	5—alfalfa meal***	15.56	49.6	8.0	396.8	325.9±11.1	55.0	25.0
11	10—alfalfa meal***	15.56	125.7	8.0	1005.6	430.0±20.2	20.0	10.0
12	3—dried pork liver	....	....	....	....	612.6± 9.1	5.0	5.0

*The per cent of the total number that developed leg paralysis during the experiment.
†The per cent of the total number that recovered before the end of the experiment.
‡See Table 2. Clover cut June 17; 96 hours' exposure.
§Timothy cut June 9 (heads emerging). Cured in sun.
‖Cut late-bloom stage.
**Meals from hays grown in Colorado.
***Meals from hays grown in Ohio.

SUMMARY AND CONCLUSIONS

Alfalfa, clover, and timothy hays cut at different times and cured under different conditions were assayed for vitamin B_1 and G with rats. The results show that these hays contain significantly more vitamin G than B_1. The vitamin B_1 and G contents of the hays decreased as the plant matured and, in general, were correlated with leafiness, greenness, and protein content of the plant.

The exposure of alfalfa to weather (day and night) for 96 hours, over one-half of which was sunshine without rain, did not affect the vitamin G content. Rain (0.68 inch), on the contrary, removed as much as 50 per cent of this factor.

Timothy and clover cut early may have as high a vitamin G content as alfalfa cut later with a much greener color.

Bluegrass, rye, and wheat compare favorably with alfalfa and clover, when cut at similar stages of maturity, in their protein and vitamin G content.

The method of testing for vitamin G by growth and incidence of leg paralysis in chicks compared favorably with the rat assay method.

It required 10 per cent of an alfalfa leaf meal containing 13 rat units of vitamin G per gram to induce good growth in chicks and prevent the occurrence of leg paralysis.

Any method or procedure of producing hay that would lower the leaf content (per cent) would lower the vitamin G content of the hay.

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